

**AMENDMENT**

**In The Claims:**

Please amend the claims as follows:

1. (currently amended)        A thrombin derivative comprising an A chain and a B chain, wherein the B chain has an amino acid sequence in which ~~[[one]]~~ two or more kinds of active center amino acids selected from the group consisting of serine at position 205, glycine at position 203, aspartic acid at position 99, and histidine at position 43 in the amino acid sequence of a thrombin B chain are substituted, and wherein

(1) the thrombin derivative cleaves a thrombin substrate at the ratio of 10% or less when it is reacted with the thrombin substrate in 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl at 37°C for 3 hours, and

(2) the thrombin derivative maintains a structure of exosite I.

2. (currently amended)        A thrombin derivative comprising an A chain and a B chain, wherein the B chain has an amino acid sequence in which ~~[[one]]~~ two or more kinds of active center amino acids selected from the group consisting of serine at position 205, glycine at position 203, aspartic acid at position 99, and histidine at position 43 in the amino acid sequence of a thrombin B chain are substituted, wherein

(1) the thrombin derivative cleaves a thrombin substrate at the ratio of 10% or less when it is reacted with the thrombin substrate in 50 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl at 37°C for 3 hours, and

(2) the thrombin derivative maintains a binding ability to hirudin C-terminal peptide-immobilized gel.

3. (original) The thrombin derivative according to claim 2, further maintaining a binding ability to heparin.

4. (previously presented) The thrombin derivative according to claim 1, wherein the thrombin substrate is a blood coagulation factor 13.

5. (previously presented and withdrawn) The thrombin derivative according to claim 1, wherein the thrombin substrate is fibrinogen.

**Claims 6 and 7 (canceled)**

8. (previously presented) The thrombin derivative according to claim 1, wherein the substitution of the active center amino acids comprises substitutions of serine at position 205 and histidine at position 43.

9. (original) The thrombin derivative according to claim 8, wherein the histidine at position 43 is substituted by alanine or serine.

10. (currently amended) The thrombin derivative according to claim [[6]] 1, wherein the serine at position 205 is substituted by alanine, threonine, or glycine.

11. (currently amended) The thrombin derivative according to claim [[6]] 1, wherein the serine at position 205 is substituted by alanine.

12. (original) The thrombin derivative according to claim 8, wherein the serine at position 205 and the histidine at position 43 are substituted by alanine.

13. (previously presented) The thrombin derivative according to claim 1, wherein its blood coagulation factor 8-binding ability is 10% or more of that of anhydrothrombin.

14. (previously presented) The thrombin derivative according to claim 1, wherein its blood coagulation factor 8-binding ability is 80% or more of that of anhydrothrombin.

15. (previously presented) The thrombin derivative according to claim 1, wherein  $VIIIa/FA$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a fibrinogen-binding ability ( $FA$ ) of the thrombin derivative is 1.1 folds or more of  $VIIIa/Fa$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a fibrinogen-binding ability ( $Fa$ ) of anhydrothrombin.

16. (previously presented) The thrombin derivative according to claim 1, wherein  $VIIIa/FA$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a fibrinogen-binding ability ( $FA$ ) of the thrombin derivative is 1.2 folds or more of  $VIIIa/Fa$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a fibrinogen-binding ability ( $Fa$ ) of anhydrothrombin.

17. (previously presented) The thrombin derivative according to claim 1, wherein an activated partial thromboplastin time of the thrombin derivative is 1.1 folds or more of that of anhydrothrombin.

18. (previously presented) The thrombin derivative according to claim 1, wherein its fibrinogen-binding ability has been decreased by 10% or more and its activated partial thromboplastin time has been prolonged 1.1 folds or more owing to the amino acid substitutions of the active center amino acids.

19. (original) A thrombin derivative comprising an A chain and a B chain, wherein the B chain has an amino acid sequence in which serine at position 205 and one or more kinds of amino acids selected from the group consisting of glycine at position 203, aspartic acid at position 99, and histidine at position 43, in the amino acid sequence of a thrombin B chain are substituted.

20. (original) A thrombin derivative comprising an A chain and a B chain, wherein the B chain has an amino acid sequence in which serine at position 205 and histidine at position 43 in the amino acid sequence of a thrombin B chain are substituted.

21. (previously presented and withdrawn) The thrombin derivative according to claim 1, wherein the B chain has an amino acid sequence in which amino acids except the active center amino acids are further substituted.

22. (withdrawn) The thrombin derivative according to claim 21, wherein the amino acids except the active center amino acids are amino acids in an exosite I region of thrombin.

23. (withdrawn) The thrombin derivative according to claim 22, wherein the amino acids in the exosite I region of thrombin are basic amino acids.

24. (withdrawn) The thrombin derivative according to claim 22, wherein the amino acids in the exosite I region of thrombin are one or more amino acids selected from the group consisting of glutamine at position 24, lysine at position 65, and lysine at position 77 in a thrombin B chain.

25. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein  $VIII A/FA$ , a ratio between a blood coagulation factor 8-binding ability (VIII A) and a

fibrinogen-binding ability (FA) of the thrombin derivative is 1.1 folds or more of VIIIa/Fa, a ratio between a blood coagulation factor 8-binding ability (VIIIa) and a fibrinogen-binding ability (Fa) of a thrombin derivative before the amino acids except the active center amino acids are substituted.

26. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein VIIIa/FA, a ratio between a blood coagulation factor 8-binding ability (VIIIa) and a fibrinogen-binding ability (FA) of the thrombin derivative is 1.5 folds or more of VIIIa/Fa, a ratio between a blood coagulation factor 8-binding ability (VIIIa) and a fibrinogen-binding ability (Fa) of a thrombin derivative before the amino acids except the active center amino acids are substituted.

27. (previously presented and withdrawn) The thrombin derivative according to claim 21, said thrombin derivative having:

any one of the effects selected from the group consisting of an APTT-prolonging effect, a thrombin receptor activation-inhibiting effect, and a ristocetin-induced platelet aggregation-inhibiting effect; and having

a decreased thrombomodulin-binding ability as compared to that of a thrombin derivative before the amino acids except the active center amino acids are substituted.

28. (previously presented and withdrawn) The thrombin derivative according to claim 21, said thrombin derivative having:

any one of the effects selected from the group consisting of an APTT-prolonging effect, a thrombin receptor activation-inhibiting effect, and a ristocetin-induced platelet aggregation-inhibiting effect; and having

a thrombomodulin-binding ability decreased by 10% or more as compared to that of a thrombin derivative before the amino acids except the active center amino acids are substituted.

29. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein  $VIIIa/TMA$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a thrombomodulin-binding ability ( $TMA$ ) of the thrombin derivative is 1.1 folds or more of  $VIIIa/TMa$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a thrombomodulin-binding ability ( $TMa$ ) of a thrombin derivative before the amino acids except the active center amino acids are substituted.

30. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein  $VIIIa/TMA$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a thrombomodulin-binding ability ( $TMA$ ) of the thrombin derivative is 1.5 folds or more of  $VIIIa/TMa$ , a ratio between a blood coagulation factor 8-binding ability ( $VIIIa$ ) and a thrombomodulin-binding ability ( $TMa$ ) of a thrombin derivative before the amino acids except the active center amino acids are substituted.

31. (withdrawn) The thrombin derivative according to claim 21, wherein the amino acids except the active center amino acids are amino acids in an exosite II region of thrombin, and its antithrombotic ability is maintained while its heparin-binding ability is decreased.

32. (withdrawn) The thrombin derivative according to claim 31, wherein the amino acids in the exosite II of thrombin are one or more kinds of amino acids selected from the group consisting of arginine at position 98, arginine at position 245, lysine at position 248, and lysine position 252 in a thrombin B chain.

33. (previously presented and withdrawn) The thrombin derivative according to claim 31, wherein the heparin-binding ability of the thrombin derivative is 90% or less as compared to that of a thrombin before the amino acids except the active center amino acids are substituted.

34. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein one or more kinds of antithrombotic effects selected from the group consisting of an activated partial thromboplastin time-prolonging effect, an modified thrombin-induced platelet aggregation-inhibiting effect, and a ristocetin-induced platelet aggregation-inhibiting effect are enhanced.

35. (previously presented and withdrawn) The thrombin derivative according to claim 21, wherein the activated partial thromboplastin time of the thrombin derivative is 1.1 folds or more as compared to that of anhydrothrombin.

36. (previously presented and withdrawn) The thrombin derivative according to claim 31, wherein the activated partial thromboplastin time of the thrombin derivative is 1.5 folds or more as compared to that of a thrombin before the amino acids except the active center amino acids are substituted, and its thrombomodulin-binding ability is decreased to 50% or less as compared to that of a thrombin before the amino acids except the active center amino acids are substituted.

37. (previously presented) The thrombin derivative according to claim 1, wherein the amino acid sequence of the thrombin B chain is an amino acid sequence of a B chain of human wild-type thrombin.

38. (original) The thrombin derivative according to claim 37, wherein the amino acid sequence of the B chain of the human wild-type thrombin is an amino acid sequence from position 50 to position 308 of SEQ ID NO: 2.

39. (previously presented) The thrombin derivative according to claim 1, wherein its carboxyl group is modified.

40. (withdrawn) The thrombin derivative according to claim 39, wherein the carboxyl group is modified by an ester of an amino acid.

41. (withdrawn) The thrombin derivative according to claim 39, wherein the carboxyl group is modified by polyethylene glycol.

42. (original) The thrombin derivative according to claim 39, wherein the carboxyl group is modified by polyethylene glycol having an amino group.

43. (previously presented) The thrombin derivative according to claim 41, wherein the polyethylene glycol is polyethylene glycol having a molecular weight of 1,000 or less.

44. (withdrawn) The thrombin derivative according to claim 39, wherein the carboxyl group is modified by carbodiimide.

45. (original) The thrombin derivative according to claim 39, wherein at least 3 carboxyl groups per molecule are modified.



46. (original) The thrombin derivative according to claim 39, wherein 25 or less carboxyl groups per molecule are modified.

47. (original) The thrombin derivative according to claim 39, wherein at least a carboxyl group of glutamic acid at position 25 in the B chain is modified.

48. (previously presented) The thrombin derivative according to claim 1, having a PAR1 activation-inhibiting effect and/or a ristocetin-induced platelet aggregation-inhibiting effect.

49. (previously presented and withdrawn) A DNA encoding the thrombin derivative according to claim 1.

50. (previously presented) A pharmaceutical composition, comprising the thrombin derivative according to claim 1.

51. (withdrawn) The pharmaceutical composition according to claim 50, which is an antithrombotic agent.

52. (withdrawn) The pharmaceutical composition according to claim 50, which is an anti-inflammatory agent.

53. (withdrawn) The pharmaceutical composition according to claim 50, which is a platelet aggregation-inhibiting agent.

54. (withdrawn) The pharmaceutical composition according to claim 50, which is a platelet adhesion-inhibiting agent.

55. (withdrawn) The pharmaceutical composition according to claim 50, which is an endogenous blood coagulation-inhibiting agent.

56. (withdrawn)      The pharmaceutical composition according to claim 50, which is a thrombin receptor activation-inhibiting agent.

57. (withdrawn)      The pharmaceutical composition according to claim 50, having both an anti-blood coagulation effect and an antiplatelet effect.